Journal of Applied Botany and Food Quality 87, 80 - 86 (2014), DOI:10.5073/JABFQ.2014.087.012

[1]Department of Agronomy, Tabriz Branch, Islamic Azad University, Tabriz, Iran
[2]Department of Agronomy and Plant Breeding, University of Kurdistan, Sanandaj, Iran
[3]Department of Agronomy, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran

Response of chickpea (*Cicer arietinum* L.) to exogenous salicylic acid and ascorbic acid under vegetative and reproductive drought stress conditions

Siamak Farjam[1], Adel Siosemardeh[2], Hamdollah Kazemi-Arbat[1], Mehrdad Yarnia[1], Asad Rokhzadi[3]*

(Received September 23, 2013)

Summary

Drought stress is a critical limiting factor in plant growth. Substances such as salicylic acid (SA) and ascorbic acid (AA) may enhance drought tolerance in plants. Therefore this experiment was aimed to study the growth and physiological response of two chickpea cultivars (ILC482 & Kurdistan) to SA and AA foliar application in four different conditions of drought stress including: control or well watered, vegetative drought stress, reproductive drought stress and complete drought stress (vegetative and reproductive drought stress). Results showed that plant biomass was significantly increased through SA and AA application. SA spraying in complete drought stress condition significantly increased the proline content of leaves. Foliar application of SA and AA both in well watered and water deficit treatments reduced the electrolyte leakage in leaves. Generally it was concluded that SA and AA have the potential of diminishing injurious effects of drought stress and promoting crop productivity.

Introduction

Chickpea (*Cicer arietinum* L.) is the third most important pulse crop in the world (JALOTA et al., 2006). In Iran chickpea is the most important grain legume and more than 90% of chickpea is grown under rainfed conditions where the crop is subjected to terminal drought stress leading to high decrease in grain yield (SOLTANI et al., 2001; ANONYMOUS, 2013). Water deficiency stress can confuse plant growth through influencing cell turgor, stomatal closure and enzymatic processes which are directly under the control of water potential. Improvement of drought tolerance in plants is possible in various ways such as plant breeding techniques and application of plant growth regulators. Utilization of growth regulating substances including salicylic acid, ascorbic acid and jasmonic acid is easier and cheaper than the long-term and costly methods of plant breeding (EL-TAYEB, 2005). Salicylic acid is an antioxidant compound which prohibits the activity of reactive oxygen species (ROS) (HAYAT et al., 2010). ROS such as hydrogen peroxide, hydroxyl radical and superoxide can damage mitochondria and chloroplasts. Salicylic acid, as an endogenous phenolic growth regulator, adjusts the activity of antioxidant enzymes, enhances plant resistance to abiotic stresses and interferes in physiological processes such as stomatal conductance in plants (HAYAT et al., 2010). Salicylic acid is involved in water relations of plant cells in abiotic stress conditions and it is well known that SA diminishes the impairments arisen from water deficiency in plants (HUSSAIN et al., 2009).

Ascorbic acid is found in the cytosol, chloroplasts, vacuoles and mitochondria of plant cells. It has a great effect on physiological processes such as cell division, plant growth and the biosynthesis of cell wall, metabolites and phytohormones. Moreover ascorbic acid plays a vital role in renovation of chloroplast and mitochondrion membranes (BARTH et al., 2004; PAVET et al., 2005; BARTH et al., 2006).

ARFAN et al. (2007) reported that foliar application of salicylic acid increased grain yield and growth of spring wheat in water deficit conditions. SHIRANI BIDABADI et al. (2012) reported that banana shoot tips responded positively to the application of salicylic acid by showing significant increase in proline and chlorophyll contents under drought stress conditions. ROSALES et al. (2006) reported that ascorbic acid increased fresh weight of leaves and decreased the damages arised from free radicals under drought stress conditions. SA and AA can diminish the injuries in cell membranes through enhancing the antioxidant potential of plant during drought stress period (AVANCINI et al., 2003; ATHAR et al., 2008).

In our country, there are insufficient research studies regarding the effects of plant growth regulators on agronomic and physiological characteristics of crop plants in drought stress conditions.Therefore, this experiment was aimed to study the reaction of two chickpea cultivars to the application of two plant growth regulators (i.e salicylic acid and ascorbic acid) under drought stress conditions.

Materials and methods

Experimental site

This study was carried out during the growing season of 2010-2011 at the agricultural research station of Gerizeh, Sanandaj, west of Iran. This research station is located at latitude of 35° 16’ N and longitude of 47° 1’ E with an altitude of 1375 m above sea level. The long-term values of mean temperature and annual precipitation in this location are 13.4 °C and 491 mm respectively. The texture of soil was clay loam with the chemical properties of: pH 7.7, organic carbon 1.53 %, electrical conductivity 0.65 dS m^{-1}, available P and K, 7 and 220 ppm respectively, total N 0.14 % and TNV 10 %.

Experiment layout, plant material and growing conditions

The operations of field preparation including mouldboard ploughing and disking were done at the proper time before sowing. The trial was laid out in a split-plot factorial arrangement with randomized complete block design in four replications. Four levels of drought stress including: 1. Control (well watered, 2. Drought stress at vegetative stage (not watered from branching to 50 % flowering stage), 3. Drought stress at reproductive stage (not watered at 50 % flowering stage) and 4. Complete drought stress (not watered at vegetative and reproductive stages) were compared as main plots. The factorial combinations of cultivar (with two levels) and PGR spraying (with three levels) were placed in sub-plots. Two chickpea genotypes were: ILC482 cultivar (drought tolerant) and Kurdistan landrace (drought susceptible) and PGR spraying levels consisted of: 1. Control or spraying with distilled water 2. Salicylic acid spraying at the rate of 200 ppm, and 3. Ascorbic acid spraying at the rate of 100 ppm. Chickpea seeds were obtained from Agricultural & Natural Resources Research Center of Kurdistan, Iran. From sowing to the start of branching, all experimental plots were fully watered to achieve best establishment of the plants. Foliar spraying of the

---

* Corresponding author

plants was performed at the vegetative stage of fifth lateral branch emergence. Experimental plots consisted of planting rows 9 m in length with 25 cm space between rows and 10 cm between plants in each row. Before sowing, the chickpea seeds were treated with 2 g L^{-1} benomyl fungicide to prevent soil-borne diseases, then the seeds were hand-sown on 9 April 2011. Irrigation of experimental plots was done through drip irrigation system. Soil moisture content was measured by gravimetric method and with consideration of essential irrigation depth for chickpea, the amount of water requirement for each plot was determined. Water stress treatments were exerted at relevant developmental stages and transparent plastic covers were used to prevent rain falling on stressed plots and the covers were removed after each period of rainfall to prevent heatstroke effects on plants.

Crop traits measurement

At maturity stage, an area of 3 m^2 of central rows of each plot was hand-harvested and after separating the seeds from other parts of plant shoot, the seeds were air dried and the weight of seeds was measured by digital electric scale and converted to kg per hectare as seed yield per unit area. The remaining parts of the shoot including stems, leaves and pods shell were air dried and their total weight was recorded. The total weight of shoot plant including the weight of seeds, stems, leaves and pods shell were considered as plant biomass and converted to kg per hectare as plant biomass per unit area.

For determining the pods number per plant, 10 plants were randomly harvested from central rows of each plot and their pods were counted. Then the total number of pods produced by 10 plants was converted to pods number per one plant.

Soluble carbohydrates assessment

Determination of soluble carbohydrates was performed by the anthrone method (YEMN and WILLIS, 1954; SANCHEZ et al., 1998). The concentration of carbohydrates was estimated as mg g^{-1} using a standard curve.

Proline estimation

Estimation of proline content was performed by the ninhydrin procedure described by BATES et al. (1973). Proline concentration was determined from a calibration curve and calculated as mg proline g^{-1}.

Cell membrane damage assay

The leakage rate from the tissues of drought-stressed plants can be taken as an index of damage to plant cell membrane (SAIRAM et al., 1997). Therefore cell membrane damage was assessed by measuring the trait of electrolyte leakage (EL). Measurement of electrolyte leakage was done through the method described by NAYYAR and GUPTA (2006) and LUTTS et al. (1996). Leaf samples were washed with distilled water and placed in closed vials containing 10 ml of distilled water at 25 °C on a shaker for 24 h and subsequently electrical conductivity of the solution (L_1) was recorded. The same samples were then autoclaved at 120 °C for 20 min. After cooling the solution to room temperature, the final electrical conductivity (L_2) was recorded. The EL was calculated as: $\mathrm{EL}\ (\%) = (L_1/L_2)\times 100$.

Statistical analysis

The measured data were analyzed statistically by analysis of variance operations using the SAS computer package version 9.1 (SAS Institute, Cary, NC). Means of treatments were compared by Duncan's multiple range test at the 0.05 level of significance.

Results and discussion

Seed yield

Effects of drought stress on seed yield were statistically significant ($P \leq 0.01$). The highest rate of seed yield (952 kg ha^{-1}) was obtained by the control (fully watered) treatment. The mean seed yield of under stress plants decreased at the ratio of 61 % as compared with control plants (Tab. 1). In well watered conditions, photosynthesis rate and assimilates production are increased which consequently results in the elevation of seed yield through increasing in seed filling rate and seed weight (REZAEYAN ZADEH, 2008). Moreover comparison between the recorded yields in vegetative and reproductive stress treatments revealed that the yield reduction in reproductive stress treatment was more pronounced compared to vegetative stress, indicating the vulnerability of chickpea yield to terminal drought stress prevailing and occurring at reproductive stage of chickpea development. Terminal drought stress is considered as a primary constraint to chickpea productivity in countries such as Iran, where the crop is generally sown after the main rainy season and grown on stored soil moisture (SERRAJ et al., 2004). Reduction in chickpea seed yield by means of terminal drought stress has been previously reported by other authors (BEHBOUDIAN et al., 2001; FALLAH et al., 2005).

Chickpea cultivars were not significantly different with respect to seed yield (Tab. 1). Likewise the application of plant growth regulators did not affected the seed yield of chickpea, even though the application of ascorbic acid resulted in some improvement in seed yield as compared with other treatments of foliar spraying (Tab. 1), suggesting that AA can be considered as a beneficial plant growth regulator in field condition. Ascorbic acid is considered as one of the plant responses in drought stress conditions which is involved in detoxification of reactive oxygen species (GUO et al., 2005).

Pods number per plant

The results showed that drought stress significantly affected the pods number per plant (Tab. 1). The maximum number of pods per plant was obtained by the well watered treatment. Pods number was decreased at the rate of 54 % (in vegetative stress treatment) and 58 % (in reproductive stress treatment) comparing to control treatment. Whereas complete drought stress resulted in 66 % decrease

Tab. 1: Effects of drought stress, cultivar and plant growth regulators on seed yield, pods number per plant and biomass of chickpea.

Treatment	Seed yield (kg ha^{-1})	Pods number per plant	Plant biomass (kg ha^{-1})
Drought stress			
Control (well watered)	952.0 a	75.0 a	2328.8 a
Vegetative stress	445.0 b	34.7 b	1004.4 b
Reproductive stress	356.8 bc	31.4 b	815.4 bc
Complete stress	303.5 c	25.7 b	541.7 c
Cultivar			
ILC482	536.8 a	44.6 a	1154.5 a
Kurdistan	491.8 a	38.8 b	1190.7 a
Plant growth regulator			
Control	485.0 a	39.4 a	1027.3 b
Salicylic acid	504.4 a	40.1 a	1239.2 a
Ascorbic acid	553.5 a	45.6 a	1251.2 a

[a] Values followed by the same letters in a group of a column are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

in pods number as compared to control or fully watered treatment (Tab. 1). Similar results obtained by MAFAKHERI et al. (2010) showed that drought stress had considerable effects on the number of pods in chickpea plants. In drought stress conditions, chickpea plants are confronted with reduction in the number of flowers and pods, in addition to abortion of flowers and pods and their subsequent abscission take place, consequently the number of pods per plant will be reduced. It is reported that drought stress is one of the most important factors responsible for yield reduction in chickpea which is mostly related to pod abscission. When leaves senescence is induced as the result of drought stress, the abscission of pods will take place (SIDDIQUE and SEDGLEY, 1986).

There was a significant difference between cultivars regarding pods number per plant. The pods number recorded by the cultivar ILC482 was 15 % more than which recorded by the Kurdistan landrace (Tab. 1). In a three years study KANOUNI et al. (2003) declared that ILC482 cultivar with regard to its high seed yield and low fluctuations in yield across different years, may be introduced as a high yielding and compatible genotype in the region. SAMAN et al. (2010) by studying the effects of terminal drought stress on chickpea genotypes showed that the highest number of pods per plant was recorded by ILC482 cultivar.

Plant biomass

Analysis of variance showed that the plant biomass was significantly affected by drought stress ($P \leq 0.01$). The greatest amount of plant biomass was produced by control (fully watered) treatment. Vegetative drought stress led to 57 % reduction in biomass as compared with control, whereas the reproductive and the complete drought stress treatments resulted in 65 and 77 % reduction in biomass compared to control respectively (Tab. 1). Similarly MWANAMWENGE et al. (1999) through studying the effects of water deficit stress during floral initiation, flowering and podding on growth traits of faba bean genotypes, declared that the most reduction in dry matter production was recorded by podding stress treatment, suggesting that the plants had more opportunity to recover from the loss of dry matter production during the earlier imposed drought stress treatments as compared with terminal drought stress.

The effect of growth regulators on biomass yield was significant ($P \leq 0.05$). The application of SA and AA resulted in a remarkable increase in plant biomass compared to control plants (Tab. 1). The studies of ZEID et al. (2009) on wheat plants indicated that the application of AA diminished the injurious effects of terminal drought stress and significantly increased root and shoot dry weight of plants. HELSPER et al. (1982) reported that AA application was effective in activation of carbon fixation enzymes. Foliar application of SA in drought stress and non-stress conditions led to a significant increase in dry weight of susceptible and drought tolerant cultivars of sunflower (NOREEN and ASHRAF, 2008). SA adjusts the hormonal conditions of the plants and increases the levels of auxin and cytokinin in non-stress conditions. The application of SA on wheat plants increased the amounts of auxin and ABA and prevented the reduction of cytokinin in drought stress conditions (SHAKIROVA et al., 2003). The application of acetylsalicylic acid (ASA) and gentisic acid (GTA) or other analogous of SA elevated the leaf area and dry matter in corn and soybean (KHAN et al., 2003).

Proline content

The greatest rate of proline content was recorded in plants under complete drought stress, on the other hand the recorded content of proline in plants under vegetative drought stress was low similar to well watered or control plants (Fig. 1). Considering the fact that proline assessment in this experiment was performed at reproductive stage of the plant, the effect of vegetative drought stress has probably been ameliorated at the time of proline assessment, and accordingly proline content has been reduced and reversed to a level similar to non-stress condition. Residual water resources from the preceding year in the soil are easily absorbed by chickpea roots at the beginning of growing season, besides the mean temperatures had not risen at this period of season and chickpea plants did not confront serious drought stress during vegetative phase. On the other hand, the plant at its reproductive phase encounters more drought stress conditions as the result of mean temperature elevation, solar radiation severity, evaporation increasing, expanding of leaves area and branching rate elevation. In such condition the harmful effects of drought stress on the plant could be effectively decreased by proline accumulation. In this research proline content was expressively increased in the plants under reproductive and complete drought stress conditions (Fig. 1). Proline accumulation in plants during drought stress has been similarly reported by other authors (HARE and CRESS, 1997; VERBRUGGEN and HERMANS, 2008; SZABADOS and SAVOURE, 2010). INEZ and MONTAGU (1995) also reported that proline accumulation in plant cells will result in preservation of turgor pressure and reduction of plasma membrane damage, hence osmotic adjustment is counted as an adaptive mechanism in drought tolerance. It is reported that proline content of leaves at both vegetative and reproductive stages in drought sensitive and tolerant chickpea cultivars may be increased (MAFAKHERI et al., 2010). Even though CHIANG and DANDEKAR (1995) stated that proline accumulation resulted from drought stress conditions is more prevalent at the flowering stage than the vegetative stage. However the proline content depends on plant phenology, and the position and age of leaves.

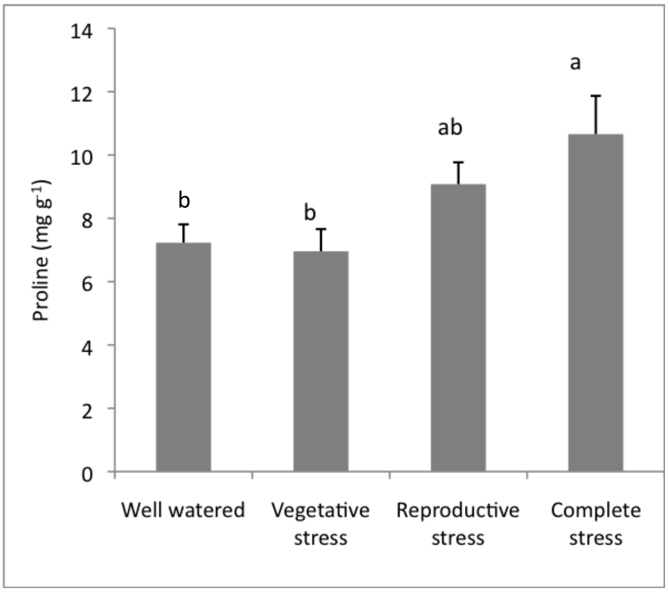

Fig. 1: Effect of drought stress on proline content of chickpea leaves. Vertical bars indicate the standard error of means. Mean values with the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

The proline accumulation rate in Kurdistan landrace was significantly higher than what was recorded for the ILC482 cultivar. Interaction effects of drought stress and genotype on proline content of leaves showed that the rate of proline accumulation by Kurdistan cultivar in all levels of drought stress was higher than ILC482 cultivar (Fig. 2). Moreover, complete drought stress led to a remarkable increase of proline content in Kurdistan cultivar. In contrast to that, the proline content of ILC482 at the complete drought stress level remained almost constant compared to the reproductive stage drought stress (Fig. 2). Considering the higher tolerance of ILC482 to drought stress with having lower content of proline, it is suggested that proline may be an appropriate indicator for assessing drought tolerance in chickpea. Similarly, other authors (Hanson et al., 1979; Premachandra et al., 1995; Sundaresan and Sudhakaran, 1995) have reported the occurrence of high proline accumulation in susceptible cultivars. It is reported that with increasing the duration and severity of drought stress, the proline content is increased depending on the plant species (Larcher, 2001). Proline is known as a beneficial solute which is accumulated in many plant species during water limitation and other stress conditions (Verslues and Sharma, 2010) that plays several roles such as: osmotic adjustment, osmoprotection, free radical scavenging and protection of macromolecules from denaturation (Vandruscolo et al., 2007).

Ascorbic acid application had no effects on proline content in comparison with control at all levels of drought stress, but salicylic acid spraying resulted in a significant increase in proline concentration of chickpea leaves in complete drought stress treatment (Fig. 3). Shakirova et al. (2003) reported that salicylic acid application increased the accumulation of proline and preservative proteins in water deficit stress conditions. Baghizadeh et al. (2009) showed that salicylic acid application accumulated more proline in the leaves of okra in comparison to ascorbic acid application. Salicylic acid has a protective role and induces high proline accumulation under drought stress conditions (Umebese et al., 2009).

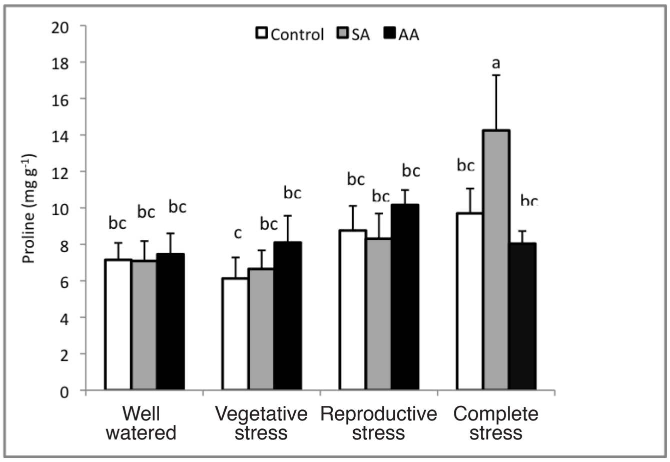

Fig. 3: Interaction effects of drought stress and plant growth regulators on proline content of chickpea leaves. Vertical bars indicate the standard error of means. Mean values with the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

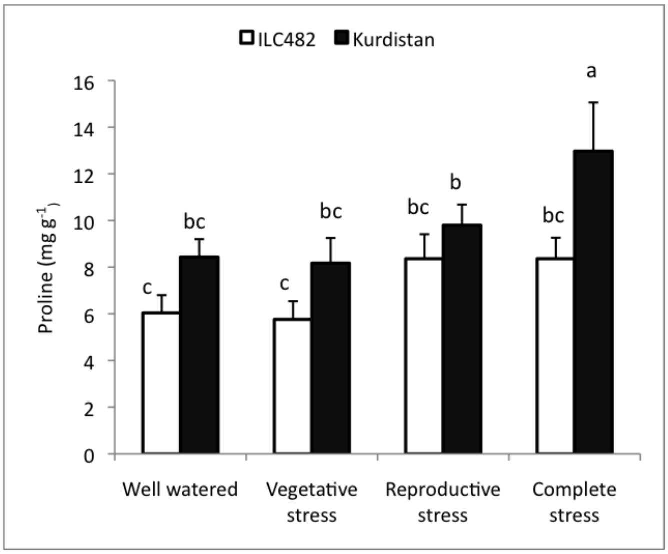

Fig. 2: Interaction effects of drought stress and genotype on proline content of chickpea leaves. Vertical bars indicate the standard error of means. Mean values with the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

Soluble Carbohydrates

Means comparison of the effects of drought stress treatments on the content of soluble carbohydrates of leaves indicated that the content of soluble carbohydrates was decreased by drought stress. The lowest concentration of soluble carbohydrates was recorded in reproductive drought stress treatment (Fig. 4). Cruz-Aguado et al. (2000) reported that soluble carbohydrates content was decreased at terminal stages of crop development in an experiment on wheat plants. In drought stress conditions, plants generally confront a decrease in carbohydrates arising from the reduction of photosynthesis rate and elevation of respiration rate (Blum, 1998). Soluble carbohydrates content in salicylic acid application treatment was significantly lower than control and ascorbic acid treatment (Fig. 5). Similarly Bakry et al. (2012), through studying the effects of foliar application of SA on two flax varieties, showed that soluble sugars content in one of the studied varieties of flax plant was significantly decreased as the result of SA foliar application, compared with untreated plants, on the other hand SA spraying caused significant increases in polysaccharides and total carbohydrates. Khodary (2004) and Ghafiyesanj et al. (2013) also declared that exogenous application of SA on maize and wheat plants significantly reduced soluble sugar content and vice versa elevated the polysaccharides and insoluble sugars content. It has been proposed that SA treatment probably activate the consumption of soluble carbohydrates to form new cell constituents as a mechanism to promote plant growth (Khodary, 2004).

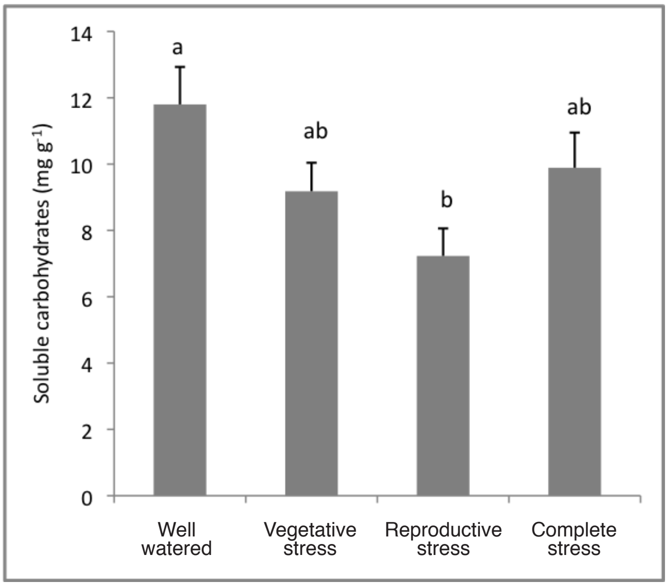

Fig. 4: Effect of drought stress on soluble carbohydrates of chickpea leaves. Vertical bars indicate the standard error of means. Mean values with the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

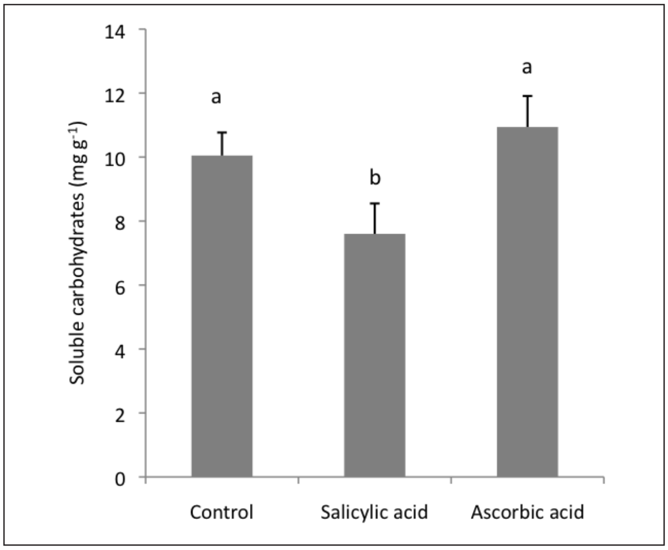

Fig. 5: Effect of plant growth regulators on soluble carbohydrates of chickpea leaves. Vertical bars indicate the standard error of means. Mean values with the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

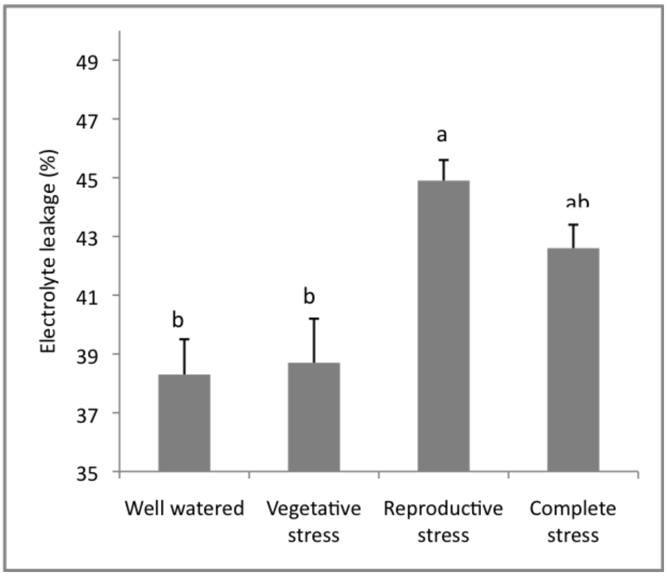

Fig. 6: Effect of drought stress on electrolyte leakage. Vertical bars indicate the standard error of means. Mean values with the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

Cell membrane damage

The highest rates of damage in cell membrane (as electrolyte leakage) were recorded by reproductive and complete drought stress treatments respectively (Fig. 6). Assessment of cell membrane damage was performed at reproductive stage of chickpea plants, therefore the possible damage to cell membrane under vegetative drought stress treatment, have probably been alleviated at the time of measurement and reached to a level similar to control treatment. Sairam et al. (1997) similarly showed that drought stress decreased the index of membrane stability of wheat genotypes at anthesis stage. Membrane damage is suggested as a consequent incident caused by reactive oxygen species (Alscher et al., 1997; Becana et al., 1998). Water deficit conditions can result in oxidative stress leading to increasing lipid peroxidation which will be subsequently terminated to membrane injury (Sairam and Saxena, 2000).

Foliar application of PGRs both in well watered and water deficit treatments resulted in the reduction of electrolyte leakage in chickpea leaves (Fig. 7). The lowest rate of electrolyte leakage was recorded by AA application in non-stressed (well watered) condition. The positive effects of SA and AA on cell membranes at vegetative stress condition was similar, however at reproductive stress condition the protective effect of AA was more remarkable than that of SA (Fig. 7). In the present study, electrolyte leakage in well watered chickpea plants was dramatically decreased as the result of AA application. In non-stressed conditions, the cell membranes are usually protected from free radical injuries, but the spring sown chickpea plants in spite of receiving enough irrigation water may confront drought stress at the terminal crop growth stage because of coinciding with high temperatures. In such conditions the application of ascorbic acid may enhance the membrane stability by its antioxidant properties (Khan et al., 2003; Guo et al., 2005).

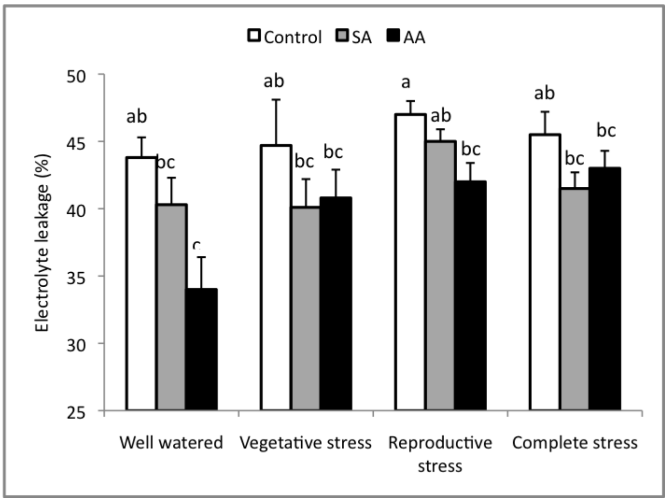

Fig. 7: Interaction effects of drought stress and plant growth regulators on electrolyte leakage. Vertical bars indicate the standard error of means. Mean values with the same letter are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

Conclusion

According to the results of this study, the reduction of yield parameters by reproductive stress was more pronounced as compared to vegetative stress, indicating the susceptibility of crop growth and yield to terminal drought stress conditions. Plant biomass was significantly increased through SA and AA foliar spraying. Supplying the plants with SA in complete drought stress condition resulted in a high accumulation of proline in chickpea leaves. The recorded rate of proline accumulation by ILC482 cultivar in all levels of drought stress was lower than Kurdistan cultivar, indicating the higher tolerance of ILC482 to drought stress. Electrolyte leakage in leaves tissue as a sign of cell membrane damage was decreased by foliar application of PGRs both in well watered and drought stress treatments, even though the positive effects of AA on cell membrane stability was more expressive. It is generally concluded that SA and AA may enhance the growth of chickpea because of their protective traits, and it could be suggested that the application of these PGRs in stressed plants will resulted in diminishing the injurious effects of drought stress and consequently the promotion of crop productivity. However, more studies about their modes of action in various field conditions are needed.

References

ALSCHER, R.G., DONAHUE, J.L., CRAMER, C.L., 1997: Reactive oxygen species and antioxidants: Relationships in green cells. Physiol. Plant. 100, 224-233.

ANONYMOUS, 2013: Agricultural statistics, Agricuture Ministry, Tehran, Iran.

ARFAN, M., ATHAR, H.R., ASHRAF, M., 2007: Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress? J. Plant Physiol. 164, 685-694.

ATHAR, H.U.R., KHAN, A., ASHRAF, M., 2008: Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. Environ. Exp. Bot. 63, 224-231.

AVANCINI, G., ABREU, I.N., SALDANA, M.D.A., MOHAMED, R.S., MAZZAFERA, P., 2003: Induction of pilocarpine formation in jaborandi leaves by salicylic acid and methyljasmonate. Phytochem. 63, 171-175.

BAGHIZADEH, A., GHORBANLI, M., HAJ MOHAMMAD REZAEI, M., MOZAFARI, H., 2009: Evaluation of interaction effect of drought stress with ascorbate and salicylic acid on some of physiological and biochemical parameters in Okra (*Hibiscus esculentus* L.) Res. J. Biol. Sci. 4, 380-387.

BAKRY, B.A., EL-HARIRI, D.M., SADAK, M.S., EL-BASSIOUNY, H.M.S., 2012: Drought stress mitigation by foliar application of salicylic acid in two linseed varieties grown under newly reclaimed sandy soil. J. Appl. Sci. Res. 8, 3503-3514.

BARTH, C., MOEDER, W., KLESSIG, D.F., CONKLIN, P.L., 2004: The timing of senescence and response to pathogens is altered in the ascorbate-deficient Arabidopsis mutant vitamin C-1. Plant Physiol. 134, 1784-1792.

BARTH, C., DE TULLIO, M., CONKLIN, P.L., 2006: The role of ascorbic acid in the control of flowering time and the onset of senescence. J. Exp. Bot. 57, 1657-1665.

BATES, L.S., WALDREN, R.P., TEARE, I.D., 1973: Rapid determination of free proline for water stress studies. Plant Soil 39, 205-207.

BECANA, M., MORAN, J.F., ITURBE-ORMAETXE, I., 1998: Iron-dependent oxygen free radical generation in plants subjected to environmental stress: toxicity and antioxidant protection. Plant Soil 201, 137-147.

BEHBOUDIAN, M.H., MA, Q., TURNER, N.C., PALTA, J., 2001: Reactions of chickpea to water stress: yield and seed composition. J. Sci. Food Agric. 81, 1288-1291.

BLUM, A., 1998: Improving wheat grain filling under stress by stem reserve mobilisation. Euphytica 100, 77-83.

CHIANG, H.H., DANDEKAR, A.M., 1995: Regulation of proline accumulation in *Arabidopsis thaliana* (L.) Heynh during development and in response to desiccation. Plant Cell Environ. 18, 1280-1290.

CRUZ-AGUADO, J.A., RODES, R., PEREZ, I.P., DORADO, M., 2000: Morphological characteristics and yield components associated with accumulation and loss of dry mass in the internodes of wheat. Field Crops Res. 66, 129-139.

EL-TAYEB, M.A., 2005: Response of barley grains to the interactive effect of salinity and salicylic acid. Plant Growth Regul. 45, 215-224.

FALLAH, S., EHSANZADEH, P., DANESHVAR, M., 2005: Grain yield and yield components in three chickpea genotypes under dryland conditions with and without supplementary irrigation at different plant densities in Khorram-Abad, Lorestan. Iran. J. Agric. Sci. 36, 719-731 (In Persian).

GHAFIYEHSANJ, E., DILMAGHANI, K., HEKMAT SHOAR, H., 2013: The effects of salicylic acid on some of biochemical characteristics of wheat (*Triticum aestivum* L.) under salinity stress. Ann. Biol. Res. 4, 242-248.

GUO, Z., TAN, H., ZHU, Z., LU, S., ZHOU, B., 2005: Effect of intermediates on ascorbic acid and oxalate biosynthesis of rice and in relation to its stress resistance. Plant Physiol. Biochem. 43, 955-962.

HANSON, A.D., NELSEN, C.E., PEDERSEN, A.R., EVERSON, E.H., 1979: Capacity for proline accumulation during water stress in barley and its implications for breeding for drought resistance. Crop Sci. 19, 489-493.

HARE, P.D., CRESS, W.A., 1997: Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Regul. 21, 79-102.

HAYAT, Q., HAYAT, S., IRFAN, M., AHMAD, A., 2010: Effect of exogenous salicylic acid under changing environment: A review. Environ. Exp. Bot. 68, 14-25.

HUSSAIN, M., MALIK, M.A., FAROOQ, M., KHAN, M.B., AKRAM, M., SALEEM, M.F., 2009: Exogenous glycinebetaine and salicylic acid application improves water relations, allometry and quality of hybrid sunflower under water deficit conditions. J. Agron. Crop Sci. 195, 98-109.

HELSPER, J.P., KAGAN, L., HILBY, C.L., MAYNARD, T.M., LOEWUS, F.A., 1982: L-Ascorbic acid biosynthesis in *Ochromonas danica*. Plant physiol. 69, 465-468.

INZÉ, D., VAN MONTAGU, M., 1995: Oxidative stress in plants. Curr. Opin. Biotechnol. 6, 153-158.

JALOTA, S.K., SOOD, A., HARMAN, W.L., 2006: Assessing the response of chickpea (*Cicer arietinum* L.) yield to irrigation water on two soils in Punjab (India): A simulation analysis using the CROPMAN model. Agric. Water Manage. 79, 312-320.

KANOUNI, H., AHMADI, M.K., SABAGHPOUR, S.H., MALHOTRA, R.S., KETATA, H., 2003: Evaluation of spring sown Chickpea (*Cicer arietinum* L.) varieties for drought tolerance. International Chickpea Conference 2003, Raipour, Chattisgarh, India.

KHAN, W., PRITHIVIRAJ, B., SMITH, D.L., 2003: Photosynthetic responses of corn and soybean to foliar application of salicylates. J. Plant Physiol. 160, 485-492.

KHODARY, S.E.A., 2004: Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt stressed maize plants. Int. J. Agric. Biol. 6, 5-8.

LARCHER, W., 2001: Physiological plant ecology. Springer Verlag Berlin Heidelberg Germany.

LUTTS, S., KINET, J.M., BOUHARMONT, J., 1996: NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Ann. Bot. 78, 389-398.

MAFAKHERI, A., SIOSEMARDEH, A., BAHRAMNEJAD, B., STRUIK, P.C., SOHRABI, Y., 2010: Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Aust. J. Crop Sci. 4, 580-585.

MWANAMWENGE, J., LOSS, S.P., SIDDIQUE, K.H.M., COCKS, P.S., 1999: Effect of water stress during floral initiation, flowering and podding on the growth and yield of faba bean (*Vicia faba* L.). Eur. J. Agron. 11, 1-11.

NAYYAR, H., GUPTA, D., 2006: Differential sensitivity of C3 and C4 plants to water deficit stress: Association with oxidative stress and antioxidants. Environ. Exp. Bot. 58, 106-113.

NOREEN, S., ASHRAF, M., 2008: Alleviation of adverse effects of salt stress on sunflower (*Helianthus annuus* L.) by exogenous application of salicylic acid: Growth and photosynthesis. Pak. J. Bot. 40, 1657-1663.

PAVET, V., OLMOS, E., KIDDLE, G., MOWLA, S., KUMAR, S., ANTONIW, J., ALVAREZ, M.E., FOYER, C.H., 2005: Ascorbic acid deficiency activates cell death and disease resistance response in Arabidopsis. Plant Physiol. 139, 1291-1303.

PREMACHANDRA, G.S., HAHN, D.T., RHODES, D., JOLY, R.J., 1995: Leaf water relations and solute accumulation in two grain sorghum lines exhibiting contrasting drought tolerance. J. Exp. Bot. 46, 1833-1841.

REZAEYAN ZADEH, E., 2008: The effects of supplemental irrigation on yield and yield components and growth indices in three chickpea cultivar (*Cicer arietinum L.*). MSc. Thesis. University of Mashhad, Iran (In Persian with English abstract).

ROSALES, M.A., RUIZ, J.M., HERNANDEZ, J., SORIANO, T., CASTILLA, N., ROMERO, L., 2006: Antioxidant content and ascorbat metabolism in cherry tomato exocarp in relation to temperature and solar radiation. J. Sci. Food Agric. 86, 1545-1551.

SAIRAM, R.K., DESHMUKH, P.S., SHUKLA, D.S., 1997: Tolerance of drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. J. Agron. Crop Sci. 178, 171-178.

SAIRAM, R.K., SAXENA, D.C., 2000: Oxidative stress and antioxidants in wheat genotypes: possible mechanism of water stress tolerance. J. Agron. Crop Sci. 184, 55-61.

Saman, M., Sepehri, A., Ahmadvand, G., Sabbaghpour, S., 2010: Effects of terminal drought stress on yield and yield components of five chickpea cultivars. Iran. J. Field Crops Sci. 2, 259-269.

Sanchez, F.J., Manzanares, M., De Andres, E.F., Tenorio, J.L., Ayerbe, L., 1998: Turgor maintenance, osmotic adjustment and soluble sugar and proline accumulation in 49 pea cultivars in response to water stress. Field Crops Res. 59, 225-235.

Serraj, R., Krishnamurthy, L., Kashiwagi, J., Kumar, J., Chandra, S., Crouch, J.H., 2004: Variation in root traits of chickpea (*Cicer arietinum* L.) grown under terminal drought. Field Crops Res. 88, 115-127.

Shakirova, M.F., Sakhabutdinova, A.R., Bezrukova, M.V., Fatkhutdinova, R.A., Fatkhutdinova, D.R., 2003: Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. Plant Sci. 164, 317-322.

Shirani Bidabadi, S., Mahmood, M., Baninasab, B., Ghobadi, C., 2012: Influence of salicylic acid on morphological and physiological responses of banana (*Musa acuminata* cv. 'Berangan', AAA) shoot tips to *in vitro* water stress induced by polyethylene glycol. Plant Omics 5, 33-39.

Siddique, K.H.M., Sedgley, R.H., 1986: Chickpea (*Cicer arietinum* L.), a potential grain legume for southwestern Australia: Seasonal growth and yield. Aust. J. Agric. Res. 37, 245-261.

Soltani, A., Khooie, F.R., Ghassemi-Golezani, K., Moghaddam, M., 2001: A simulation study of chickpea crop response to limited irrigation in a semiarid environment. Agric. Water Manage. 49, 225-237.

Sundaresan, S., Sudhakaran, P.R., 1995: Water stress-induced alterations in the proline metabolism of drought-susceptible and -tolerant cassava (*Manihot esculenta*) cultivars. Physiol. Plant. 94, 635-642.

Szabados, L., Savoure, A., 2010: Proline: a multifunctional amino acid. Trends Plant Sci. 15, 89-97.

Umebese, C.E., Olatimilehin, T.O., Ogunsusi, T.A., 2009: Salicylic acid protects nitrate reductase activity, growth and proline in amaranth and tomato plants during water deficit. Am. J. Agric. Biol. Sci. 4, 224-229.

Vendruscolo, E.C.G., Schuster, I., Pileggi, M., Scapim, C.A., Molinari, H.B.C., Marur, C.J., Vieira, L.G.E., 2007: Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat. J. Plant Physiol. 164, 1367-1376.

Verbruggen, N., Hermans, C., 2008: Proline accumulation in plants: a review. Amino Acids 35, 753-759.

Verslues, P.E., Sharma, S., 2010: Proline metabolism and its implications for plant-environment interaction. Arabidopsis Book Vol. 8.

Yemn, E.W., Willis, A.J., 1954: The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57, 508-514.

Zeid, F.A., El Shihy, O., Ghallab, A.E.M., Ibrahim, F.E.A., 2009: Effect of exogenous ascorbic acid on wheat tolerance to salinity stress conditions. Arab J. Biotechnol. 12, 149-174.

Address of the corresponding author:
Dr. Asad Rokhzadi, Department of Agronomy, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran
E-mail: asadrokh@yahoo.com